

9. On the Reproductive Organs of the Ascidian *Kükenthalia borealis* (Gottschaldt). By Dr. AUGUSTA ÄRNBÄCK-CHRISTIE-LINDE *.

(Text-figures 1-8.)

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The subfamily of the *Polyzoinae*, to which *Kükenthalia borealis* (Gottschaldt) has been referred, is regarded as an intermediate group between the *Styelidae* and the *Botryllidae*. According to Michaelsen †, who has given descriptions of this group of Ascidians in several papers, it comprises about ten genera, distributed in almost every region all over the world.

In the Arctic region it is represented by a single genus with one species, *Kükenthalia borealis*.

The species was first described by Gottschaldt ‡ under the name of *Goodsiria borealis*. It was re-described by Hartmeyer § in 'Fauna Arctica' under the new generic name *Kükenthalia*.

As it thus appears, the species has been the object of thorough examination by several eminent zoologists. However, it has not hitherto been possible, so far as I know, to make out its internal structure, owing to the unsatisfactory knowledge of the reproductive organs.

Gottschaldt (*l. c.*) merely writes as follows: "Die Geschlechtsorgane liegen in der Tunica (es wurde ein einziges ziemlich grosses Ei gefunden)."

Two years later Bonnevie || described a few colonies of the species which had been dredged by the Norwegian Atlantic Expedition, 1876-1878. They were described under the name of *Goodsiria coccinea* (Cunningham). With regard to the reproductive organs, the author states that they "are found in small polycarps in the muscular stratum," a statement which proves to be a mistake.

Hartmeyer writes (*l. c.* p. 260):—"Weiblicher Geschlechtsapparat diffus (grössere und kleinere Eizellen überall in den Innenkörper eingestreut sowie in den Blutbahnen der Darmwandung und der jungen Knospen); männlicher Geschlechtsapparat bisher nicht nachgewiesen." "Ein besonderes Ovarium ist nach unseren Untersuchungen nicht vorhanden" (*l. c.* p. 258).

In the paper of Michaelsen we find the same statement.

* Communicated by Dr. F. A. BATHER, F.R.S., F.Z.S.

† Michaelsen, W., "Revision der compositen Styeliden oder Polyzoinen." Mitteilungen aus d. Naturhist. Museum in Hamburg, Jahrgang 21. Hamburg, 1901.

‡ Gottschaldt, R., "Die Synascidien der Bremer Expedition nach Spitzbergen im Jahre 1889." Jen. Z. vol. xxviii. Jena, 1894.

§ Hartmeyer, R., "Die Ascidien der Arctis." Fauna Arctica, Bd. iii. Jena, 1903-1904.

|| Bonnevie, K., "*Ascidiae simplices* and *Ascidiae compositae*. From the North Atlantic Expedition." The Norwegian North Atlantic Expedition, 1876-1878. Christiania, 1906.

According to this author, male gonads, as well as female ones, are unknown. And he is of opinion that, with regard to the reproductive organs, the genus in question differs from all other *Polyzoine*, and even from all other *Styelids*.

Nor did Bjerkan * succeed in enlightening us on the matter, though, as he says, he had a great mass of colonies for examination.

Being occupied with studies on the Northern and Arctic Ascidians belonging to the collections of the Swedish State Museum, I have had opportunities of examining *Kükenthalia borealis*.

My investigations of the species are not yet completed. However, as important facts concerning the reproductive organs have been established, I publish the results already arrived at, hoping that they will augment our knowledge of an organic system of which one has hitherto tried in vain to get a clear conception.

The material examined was collected off Spitzbergen, Waygat Islands, 60 fathoms, August 1861, and in Ice Fjord, Safe Harbour, 30 fathoms, June 1864. I have also had at my disposal a colony from Greenland, 65° 15' N. lat., 53° 30' W. long., collected in June 1883; depth 75 fathoms.

The specimens are preserved in alcohol.

SPICULES.

Introductorily I will here mention that small spicules occur in the test of *Kükenthalia borealis*.

As is known, the species forms colonies which are massive, usually rounded, and not very large; they are attached by a

Text-figure 1.



Kükenthalia borealis (Gottsch.).

Spicules. $\times 110$.

narrow base. The zooids are not arranged in systems, and the atrial as well as the branchial aperture of each zooid opens independently on the surface. The test of the colony is well developed. Internally and at the base it is soft and gelatinous, externally it is leathery. To this might be added also that the capsules which surround the individuals of the colony are firm and tough.

The test owes its consistency, partly at least, to the presence of the above-mentioned spicules which are scattered in it. In larger colonies the spicules are abundant, in smaller ones they seem to be less numerous.

* Bjerkan, P., "Die Ascidien des nördlichen Norwegens." Tromsø Museums Aarshefter 24-25. 1908.

They show some variability with regard to size and shape. The general plan of the structure is, however, the same. Text-figure 1 shows two varieties of spicules which occur in different colonies. From a comparison it appears that the crystals are arranged about a common point forming like a sphere, studded with irregular, short, sharp points, or they radiate forming a stellate group with rays of irregular length. Sometimes a ray is elongated like a stalk.

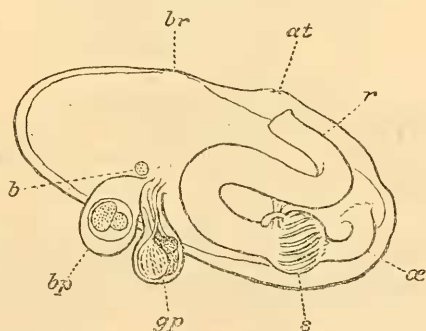
According to the literature, spicules often occur in different groups of Ascidians, but such structures do not seem to have been observed before in any genus of the *Polyzoineæ*. The possibility is, however, not excluded that they have been overlooked, since their presence has been until now unobserved in *Kükenthalia*. But if this should prove not to be the case, the spicules, having been found only in *Kükenthalia*, are to be regarded as a characteristic of the Arctic form.

As spicules might be of some value for comparative and systematic studies, an investigation of their occurrence in the different genera of *Polyzoineæ* would be of interest.

THE REPRODUCTIVE ORGANS.

A more important result of this investigation is, as will be shown, that the reproductive organs, testes as well as ovaries, have been found in *Kükenthalia borealis*.

Text-figure 2.



Kükenthalia borealis (Gottsch.).

Zooid seen from the left side, test partly removed. $\times 8$.

at. Atrial aperture. br. Branchial aperture. b. Bud. bp. Brood-pouch. gp. Genital pouch with testis and ovary. α. Esophagus. r. Rectum. s. Stomach.

The organs are in the form of a hermaphrodite structure, the male glands as well as the ovary and the ducts being enclosed in a long, sac-like outgrowth of the mantle which extends into the common test. This outgrowth is here named the genital pouch.

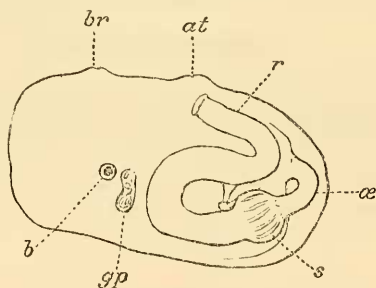
In some individuals another sac-like structure, generally of greater size, has been observed. It is situated at the upper side of the above-mentioned one, and projects like the latter into the common test. When an individual contained developing embryos, they were enclosed in this sac. No doubt it develops to receive the embryos, and it is consequently to be regarded as a brood-pouch.

In the zooids most advanced in development there are thus two pouches which are to be referred to the reproductive organs (*cf.* text-fig. 2).

In the individuals examined, reproductive organs have been observed only on the left side of the body; they are placed ventrally, on the outer side of the mantle, and are embedded in the test.

Only one genital pouch—consequently only one brood-pouch—has been observed in each zooid. If the individuals are seen from the inside of the colony, the position as well as the number of the organs in question is easily determined. The members of the

Text-figure 3.



Kükenthalia borealis (Gottsch.).

Zooid seen from the left side, test partly removed. $\times 7$.

at. Atrial aperture. *br.* Branchial aperture. *b.* Bud. *gp.* Genital pouch with testis and ovary. *æ.* Oesophagus. *r.* Rectum. *s.* Stomach.

colony are arranged in one layer and are placed close to each other side by side; only the ventral part is free. From here the reproductive organs extend between the individuals, and only one genital pouch is visible between them, projecting from the left side of each zooid.

As in the genus *Gynandrocarpa*, the reproductive organs of *Kükenthalia* seem thus to consist of a single hermaphrodite structure. In the former it is placed on the right side, in the latter on the left.

In the different zooids of the same colony the reproductive organs show various degrees of development. In some zooids the genital pouch with the gonads was very large and of considerable length, in others it was hardly distinguishable (*cf.* text-figs. 2 & 3).

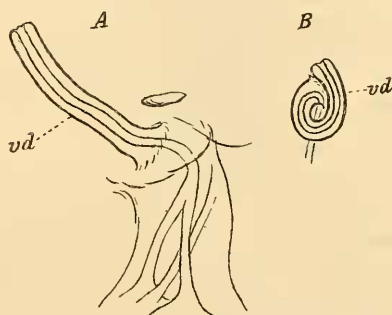
A brood-pouch was developed only in few zooids. It could not be decided whether all the zooids of the colony were mature, the material being little fit for dissection. From the above-mentioned facts one might, however, conclude that the members of the colony do not attain sexual maturity at the same time, probably owing to different age.

As appears from text-figures 2 and 3 the part of the genital pouch which contains the genital glands is of a bulb-shaped form. On one side a large swelling, enclosing the distal part of the oviduct, is visible, and its form varies, owing to the degree of development of the brood-pouch, as will be described in the following.

MALE.—The testis is composed of numerous spermatic vesicles and a long vas deferens.

As has been mentioned above, the testis is placed on the outside of the body-wall, invested by an outgrowth of the mantle, projecting into the common test. The vas deferens, which is a duct of considerable length, extends into the peribranchial cavity and opens into it.

Text-figure 4.



Kükenthalia borealis (Gottsch.).

Vas deferens. $\times 33$.

A. Vas deferens, usual form. | B. Vas deferens, coiled up.
vd. Vas deferens.

The spermatic vesicles are pyriform glands which are arranged in two bundles, on account of which the testis appears to be bipartite. The male glands open into two sperm-ducts, each bundle having its duct, and these unite as a rule into a main sperm-duct, the vas deferens (cf. text-figs. 4 A & 5).

In one individual examined another arrangement was observed. Here the two ducts did not join, but entered separately into the peribranchial cavity, thus forming two vasa deferentia.

After its entrance into the peribranchial cavity, the sperm duct

generally bends somewhat to the side, extending along the inner wall of the cavity. This arrangement was observed in all specimens but one. In the latter, a rather large individual with a strongly developed testis, the distal part of the vas deferens was coiled up (*cf.* text-fig. 4 *B*), and its opening lies close to that of the brood-pouch.

As this arrangement has been found in a single individual only, I do not venture to draw any conclusions from it. The possibility seems, however, not excluded that it might be of some importance for the fertilization, in this case probably the self-fertilization of the individual. The fact that ova and spermatozoa are not seldom found mature at the same time in the same individual appears also to support the view that self-fertilization can take place; whether it is usual is another question.

Though most Ascidians are hermaphrodite, it is, however, not probable that self-fertilization generally takes place. On the contrary, in many groups arrangements have been observed which seem to prevent it. As an instance of such an arrangement, I will mention that ova and spermatozoa are often produced at different times, and are consequently not found mature together.

From sections it appears that, in zooids belonging to colonies caught in June, masses of spermatozoa fill up the sperm-ducts. Other cells are macerated, but the spermatozoa are in good condition—a fact which ought to be noticed, the material being preserved in alcohol and dredged so many years ago. It confirms the observation made many times before that the spermatozoa are more resistant than other cells, and that they are highly resistant, even to maceration.

FEMALE.—In zooids most advanced in development the female organ consists of a small ovary with a wide oviduct and a very large brood-pouch.

As is shown by text-figure 5, a small rounded vesicle with a wide duct is situated at the side of the male glands in the genital pouch. Sections through it did not show much of its structure, the tissues being rather macerated. The presence of eggs makes it, however, evident that the vesicle is to be regarded as an ovary.

In several individuals one very large egg, covered with follicular epithelium, was observed here, apparently ready to pass through the oviduct (*cf.* text-fig. 7).

As mentioned above, the ovary is placed at the side of the male glands and is quite separated from them. Seen from the left side of the zooid, the one bundle of male glands is partly covered by the other, and the ovary has its position opposite their middle line. If one imagines the gonads spread out, the ovary would thus have its position between the two bundles of male glands.

The oviduct extends from the above-described ovary between the two sperm-ducts towards the brood-pouch, and opens into the distal part of the latter with a very wide aperture (*cf.* text-fig. 5). The lumina of the brood-pouch and the oviduct become thus

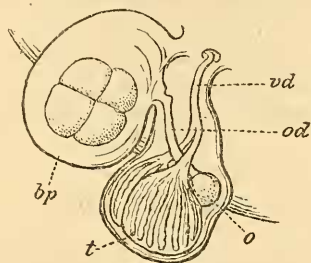
distally confluent, and they communicate with the peribranchial cavity by a short, narrow aperture which is well observable near the vas deferens (cf. text-fig. 8).

These facts are of a special interest; then, owing to the above-mentioned arrangement, the passage of the egg is secured: the oviduct opening widely into the brood-pouch, the egg, having left the oviduct, must be laid into the brood-pouch, where it probably remains until it is fully developed.

The oviduct is of considerable width, especially the distal part, which is rather sac-like, and the wall is deeply folded on one side.

Text-figure 6 represents a transverse section of the genital pouch on a level with the bifurcation of the vas deferens. The latter is on the point of forking, on account of which it appears somewhat broad. The figure shows how the oviduct is somewhat compressed from side to side; it is lined with a low epithelium.

Text-figure 5.



Kükenenthalia borealis (Gottsch.).

The reproductive organs from the outside. $\times 23$.

bp. Brood-pouch. o. Ovary. od. Oviduct. t. Testis. vd. Vas deferens.

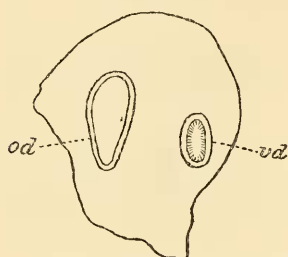
More distally, on certain parts of the wall, a strongly ciliated epithelium was observed. As appears from the figure, the ciliated epithelium of the vas deferens is also well developed.

The brood-pouch has the form of a large double-walled sac with a constricted neck. It generally contained one embryo. In some individuals it is of considerable length and width, projecting beyond the genital pouch. Text-figure 5 represents it at a stage somewhat less developed.

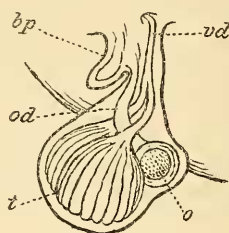
In individuals containing no embryos a brood-pouch has also been observed, though highly reduced with regard to form and size. Such an interesting stage is shown by text-figure 7, where the brood-pouch is represented by a short blind sac or an appendix in connection with the oviduct; and in zooids with rudimentary reproductive organs, as for instance in that represented by text-figure 3, the presence of such a blind sac can also be stated, as will be seen from text-figure 8. The last-mentioned

figure shows three transverse sections of the genital pouch with the ducts, which are cut obliquely. The brood-pouch is represented by a short blind sac; like the oviduct, it is compressed from side to side. In text-figure 8 *a* the oviduct and the brood-pouch

Text-figure 6.



Text-figure 7.

Text-fig. 6.—*Kükenthalia borealis* (Gottsch.).

Transverse section of the genital pouch. $\times 110$.

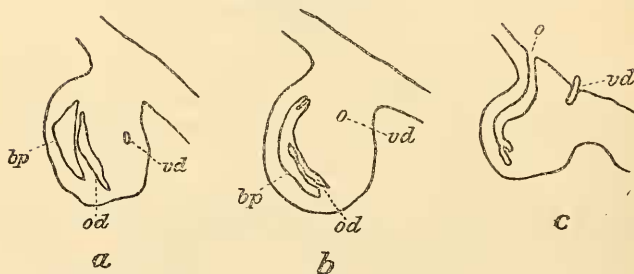
od. Oviduct. vd. Vas deferens.

Text-fig. 7.—*Kükenthalia borealis* (Gottsch.).

The reproductive organs from the outside. $\times 23$.

bp. Brood-pouch. o. Ovary. od. Oviduct. t. Testis.
vd. Vas deferens.

Text-figure 8.

*Kükenthalia borealis* (Gottsch.).

Sections through the genital pouch, showing the distal part of the oviduct and the rudimentary brood-pouch; cut obliquely. $\times 50$.

bp. Brood-pouch. od. Oviduct. o. Opening into the peribranchial cavity.
vd. Vas deferens.

are still separated; fig. *b* shows how the oviduct opens into the brood-pouch, and in fig. *c* the opening into the peribranchial cavity is visible.

Whether this blind sac is to be regarded as a vestigial trace or possibly as a rudiment of the brood-pouch, is a question which

ought to be examined in connection with that of the origin and formation of the whole complicated female organ. As it is difficult to state anything for certain about the age of the individuals hitherto examined, we cannot draw any conclusions from the above-mentioned facts as to the presence of a rudimentary brood-pouch, even before the first egg has been laid.

Another matter of interest is the question of the homology of the brood-pouch in *Kükenthalia*. Before deciding it, it ought to be investigated whether the brood-pouch of the species in question arises as a direct projection from the peribranchial cavity, or whether it is possibly formed in connection with the oviduct with which it, when fully formed, is so intimately connected.

In other Ascidians in which a brood-pouch develops to receive the embryos, it is generally a diverticulum of the peribranchial cavity.

Though the systematical position of *Kükenthalia borealis* will not be discussed in this paper, I will, however, point out that, as the structure of the reproductive organs has been made the chief character in distinguishing the genera belonging to the group *Polyzoine*, the facts which have been ascertained by this investigation will no doubt be of decisive importance for the systematic position of *Kükenthalia*. Comparing the reproductive organs of the last-mentioned genus with those of the other genera of the group, we find that the most striking difference is the presence of a brood-pouch in *Kükenthalia*. Though the *Polyzoine* have been the object of thorough investigation, especially with regard to the structure of the reproductive organs, nothing which corresponds to a brood-pouch seems to have been observed. One might thus conclude that it occurs only in the Arctic form, of which it is consequently characteristic.

With respect to the genital pouch, the beginning of analogous structures may possibly be found in some genera, though they have not attained the same degree of development as in *Kükenthalia*.

In certain respects, points of agreement may be found between *Kükenthalia* and *Gynandrocarpa*, as, for instance, they agree with regard to the reduced number of the gonads and the structure of the testis (cf. Herdman *, pl. 44. fig. 4).

NON-GONADIAL SEXUAL PRODUCTS.

Though I now venture to maintain that the presence of gonads, testis as well as ovary, in *Kükenthalia borealis* is put beyond a doubt, and that it has been proved that ova and spermatozoa are produced in special organs, there are, however, certain facts concerning the reproduction of the species which still seem

* Herdman, William A., "Report on the Tunicata collected during the voyage of H.M.S. 'Challenger' during the years 1873-76," Part 2, vol. xiv. London, 1886.

unexplained. Such a fact is, for instance, the presence of sexual products, *i. e.* ova, in the mesoderm.

Isolated ova have been observed before in the mesoderm of *Kükenthalia*, but it has not been made out where they originate. As no gonads, only isolated ova were met with, Michaelsen concluded that the female organs of *Kükenthalia* consist of ova diffusely placed in the mesoderm. Michaelsen writes as follows: "Diese Gattung weicht durch den Bau der weiblichen Geschlechtsorgane von allen übrigen *Polyzoiner*, ja, von allen *Styeliden* überhaupt, ab. Ich bezeichnete die Gestaltung desselben als 'diffus,' da die sich entwickelnden Eizellen an kein bestimmt lokalisiertes Organ gebunden sind, sondern sich weit zerstreut im Innenkörper und in den Blutbahnen anderer Organe, so besonders des Darmes, sowie in den jungen Knospen, vorfinden. Der ursprüngliche Ort der weiblichen Gonaden ist unbekannt..." (*l. c.* p. 112).

Though female organs of other structure have now been found, the observation that isolated eggs occur in the mesoderm proves to be right. According to my observation, they are, however, met with less abundantly than has been stated above. As it hardly can be thought that they originate in the above-described ovary, one must suppose that they are produced in the mesoderm, where they occur, though in no special gonads, *i. e.* non-gonadially. This supposition seems to be verified by my observation of small ovaries or, rather, groups of eggs which are situated in the mesoderm close to the wall of the peribranchial cavity and in the vicinity of bud-rudiments.

In *Kükenthalia*, buds occur on both sides of the body; on the left side they are to be seen in the vicinity of the reproductive organs above described. In the mesoderm of those bud-rudiments eggs have been observed, and even in very small bud-rudiments very large eggs are often visible. In all probability the eggs originate in the parent animal, the generative cells being supposed to wander from the latter; but the question is whether they are differentiated in the bud or have wandered at an advanced stage from the parent animal into the bud. But this is a problem which can be decided only after thorough investigation of the further development of the non-gonadal ova as well as of the formation of the reproductive organs of the bud.